Amendments to the Claims

This listing of claims will replace all prior versions, and listings of claims in the application.

1. (currently amended) A *Thermotoga maritima* (*Tma*) DNA polymerase which is modified at least two ways selected from the group consisting of:

(a) a mutation in the 3'-5' exonuclease domain of said polymerase to reduce or eliminate the 3′→5′ exonuclease activity of the polymerase;

(b) a mutation in the 5'-3' exonuclease domain of said polymerase to reduce or eliminate the 5′→3′ exonuclease activity of the polymerase; and

(c) a mutation in the O-helix of said polymerase to reduce or eliminate discriminatory behavior against a dideoxynucleotide;

wherein said mutation is selected from the group consisting of: a deletion, a single or double substitution[, a point mutation], a frame shift mutation and an insertion; wherein said *Thermotoga maritima* (*Tma*) DNA polymerase has a molecular weight of about 100 kilodaltons.

3. (original) The DNA polymerase mutant of claim 1, which is modified three ways.

5. (previously presented) The DNA polymerase of claim 1, wherein said O-helix is defined as RXXXKXXXFXXXYX (SEQ ID NO:1), wherein X is any amino acid.

6. (previously presented) The *Tma* DNA polymerase as claimed in claim 1, wherein said mutation in the O-helix is a $Phe^{730} \rightarrow Tyr^{730}$ substitution.

7. (original) The *Tma* DNA polymerase of claim 1, wherein said DNA polymerase is a *Tma* DNA polymerase having substantially reduced 3′→5′ exonuclease activity.

8. (original) The mutant *Tma* DNA polymerase as claimed in claim 7, wherein said mutant is a $Asp^{323} \rightarrow Ala^{323}$ substitution.

9. (previously presented) The mutant *Tma* DNA polymerase as claimed in claim 1, wherein the modifications are a $Phe^{730} \rightarrow Tyr^{730}$ substitution and a $Asp^{323} \rightarrow Ala^{323}$ substitution.

10. (original) The mutant DNA polymerase mutant of claim 1, wherein said DNA polymerase is a *Tma* DNA polymerase having substantially reduced 5′→3′ exonuclease activity.

13. (original) A vector comprising a gene encoding the DNA polymerase of claim 1.

16. (original) A host cell comprising the vector of claim 13.

17. (original) A method of producing a DNA polymerase, said method comprising:

(a) culturing the host cell of claim 16;

(b) expressing said gene; and

(c) isolating said DNA polymerase from said host cell.

19. (original) A method of synthesizing a double-stranded DNA molecule comprising:

(a) hybridizing a primer to a first DNA molecule; and

(b) incubating said DNA molecule of step (a) in the presence of one or more deoxy- or dideoxyribonucleoside triphosphates and the DNA polymerase of claim 1, under conditions sufficient to synthesize a second DNA molecule complementary to all or a portion of said first DNA molecule.

26. (original) A method for amplifying a double stranded DNA molecule, comprising:

(a) providing a first and second primer, wherein said first primer is complementary to a sequence at or near the 3′-termini of the first strand of said DNA molecule and said second primer is complementary to a sequence at or near the 3′-termini of the second strand of said DNA molecule;

(b) hybridizing said first primer to said first strand and said second primer to said second strand in the presence of the DNA polymerase of claim 1, under conditions

such that a third DNA molecule complementary to said first strand and a fourth DNA molecule complementary to said second strand are synthesized;

(c) denaturing said first and third strand, and said second and fourth strands; and

(d) repeating steps (a) to (c) one or more times.

28. (original) A kit for sequencing a DNA molecule, comprising:

(a) a first container means comprising the DNA polymerase of claim 1;

(b) a second container means comprising one or more dideoxyribonucleoside triphosphates; and

(c) a third container means comprising one or more deoxyribonucleoside triphosphates.

29. (original) A kit for amplifying a DNA molecule, comprising:

(a) a first container means comprising the DNA polymerase of claim 1; and

(b) a second container means comprising one or more deoxyribonucleoside triphosphates.

34. (currently amended) A method of preparing cDNA from mRNA, comprising

(a) contacting mRNA with an oligo(dT) primer or other complementary primer to form a hybrid, and

(b) contacting said hybrid formed in step (a) with the [*Tne*] DNA polymerase [or mutant] of claim 1 and dATP, dCTP, dGTP and dTTP,

whereby a cDNA-RNA hybrid is obtained.

35. (currently amended) A method of preparing dsDNA from mRNA, comprising

(a) contacting mRNA with an oligo(dT) primer or other complementary primer to form a hybrid, and

(b) contacting said hybrid formed in step (a) with the [*Tne*] DNA polymerase [or mutant] of claim 1, dATP, dCTP, dGTP and dTTP, and an oligonucleotide or primer which is complementary to the first strand cDNA;

whereby dsDNA is obtained.

36. (previously presented) A method of sequencing a DNA molecule, comprising:

(a) hybridizing a primer to a first DNA molecule;

(b) contacting said DNA molecule of step (a) with deoxyribonucleoside triphosphates, the DNA polymerase of claim 1, and a terminator nucleotide;

(c) incubating the mixture of step (b) under conditions sufficient to synthesize a random population of DNA molecules complementary to said first DNA molecule, wherein said synthesized DNA molecules are shorter in length than said first DNA molecule and wherein said synthesized DNA molecules comprise a terminator nucleotide at their 3′ termini; and

(d) separating said synthesized DNA molecules by size so that at least a part of the nucleotide sequence of said first DNA molecule can be determined.

37. (currently amended) An isolated DNA molecule encoding a *Thermotoga maritima* (*Tma*) DNA polymerase mutant which is modified at least two ways selected from the group consisting of:

(a) a mutation in the 3'-5' exonuclease domain to reduce or eliminate the 3'→5' exonuclease activity of the polymerase;

(b) a mutation in the 5'-3' exonuclease domain to reduce or eliminate the 5'→3' exonuclease activity of the polymerase; and

(c) a mutation in the O-helix to reduce or eliminate discriminatory behavior against a dideoxynucleotide;

wherein said mutation is selected from the group consisting of: a deletion, a single or double substitution, [a point mutation,] a frame shift mutation and an insertion; wherein said *Thermotoga maritima* (*Tma*) DNA polymerase has a molecular weight of about 100 kilodaltons.

38. (previously presented) A mutant *Tma* DNA polymerase having a mutation in the O-helix resulting in said DNA polymerase becoming non-discriminating against dideoxynucleotides, or a fragment of said mutant DNA polymerase said fragment having polymerase activity.

40. (previously presented) The mutant *Tma* DNA polymerase of claim 39, wherein said O-helix is defined as RXXXKXXXFXXXYX, wherein X is any amino acid.

41. (previously presented) The *Tma* polymerase of claim 40, wherein said mutation is a $Phe^{730} \rightarrow Tyr^{730}$ substitution.

42. (previously presented) A method of synthesizing a double-stranded DNA molecule comprising:

(a) hybridizing a primer to a first DNA molecule; and

(b) incubating said DNA molecule in the presence of one or more deoxy or dideoxyribonucleoside triphosphates and the DNA polymerase of claim 38, under conditions sufficient to synthesize a second DNA molecule complementary to all or a portion of said first DNA molecule.

43. (previously presented) A method of amplifying a double stranded DNA molecule, comprising:

(a) providing a first and second primer, wherein said first primer is complementary to a sequence at or near the 3'-termini of the first strand of said DNA molecule and said second primer is complementary to a sequence at or near the 3'-termini of the second strand of said DNA molecule;

(b) hybridizing said first primer to said first strand and said second primer to said second strand in the presence of a DNA polymerase of claim 38, under conditions such that

a third DNA molecule complementary to said first strand and a fourth DNA molecule complementary to said second strand are synthesized;

(c) denaturing said first and third strand, and said second and said fourth strand; and

(d) repeating (a) to (c) one or more times.

44. (currently amended) A method of sequencing a DNA molecule, comprising:

(a) hybridizing a primer to a first DNA molecule;

(b) contacting said DNA molecule of step (a) with [dexyribonucleoside] deoxyribonucleoside triphosphates, a DNA polymerase of claim 38, and a terminator nucleotide;

(c) incubating the mixture of step (b) under conditions sufficient to synthesize a random population of DNA molecules complementary to said first DNA molecule, wherein said synthesized DNA molecules are shorter in length than said first DNA molecule and wherein said synthesized DNA molecules comprise a terminator nucleotide at their 3' termini; and

(d) separating said synthesized DNA molecules by size so that at least a part of the nucleotide sequence of said first DNA molecule can be determined.